stained with Hoeschst (D) for nuclei. The formed tubes were positive for both pericyte specific markers NG2 (G) and a-SMA (FIG. **7**K) and the endothelial cell specific marker von Willebrand factor (**7**F and **7**J). FIGS. **7**H and **7**L shows von Willebrand factor and Hoeschst (**7**E and **7**I) merged with NG2 and α-SMA, respectively.

**[0020]** FIG. **8** depicts a schematic representation of the differentiation strategy of ASC. Light micrographs are shown that were taken at various days of ASC differentiated to epithelial like cells. Control (Top), differentiated ASC (bottom).

**[0021]** FIG. **9** depicts isolated ASC that were analyzed by immunohistochemistry and expressed p63.

**[0022]** FIG. **10** depicts cells that were subjected to keratinocyte differentiation and expressed a deep granulated appearance.

**[0023]** FIG. **11** depicts the staining for Pan-cytokeratin and cytokeratin 18 of cells cultured in complete media (GFs).

**[0024]** FIGS. **12**A and **12**B depict sections showing differentiated ASC to organize like an epithelial layer over collagen matrix (A; col-collagen). Immunofluorescence image of section showing positive for pan cytokeratin (B) and krt 10 (C) (Alexa fluor 594 and Hoeschst overlay).

**[0025]** FIG. **13** depicts the immunocytochemical analysis of ASC isolated from rats. Photomicrographs are of markers expressed in third passage ASC. Figures in each panel indicate the specific cell surface marker. All antibodies, except Stro-1, are FITC-labeled primary antibodies. Stro-**1** is identified using isotype matched FITC-labeled rat IgM. All photomicrographs at ×20 magnification.

**[0026]** FIG. **14** depicts light microscopic images of differentiation time-course of ASC into vascular like structures. Cells began to form vascular tube-like networks in the PEGylated fibrin gel in the absence of additional soluble cytokines. The amount of network formation was related to the initial cell density. (Scale bar=100 μm)

**[0027]** FIG. **15** depicts endothelial and pericyte specific markers expressed by the differentiated ASC in PEGylated fibrin gels. Expression levels of endothelial cell specific markers (CD31, von Willebrand factor) and pericyte specific markers (NG2 and PDGFRβ) were analyzed using Real Time Polymerase Chain Reaction (RT-PCR). There was significant increase in endothelial cell specific markers; CD31 (25 fold) and vWF (42 fold) in comparison to pericyte markers; NG2 (6 fold) and PDGFRβ (9 fold) by day 11.

**[0028]** FIGS. **16**A-**16**F are images depicting ASC released from chitosan microspheres in vitro in PEGylated fibrin and collagen gels. Phase contrast images of ASC migrated from chitosan microspheres into collagen (A, B and C) and PEGylated fibrin (D, E and F). ASC that have migrated from CSM attached to the PEGylated fibrin shows classical sprouting (A, day 2) followed by differentiating into tube-like structures (B, day 5). Over the time course of differentiation, they migrate into the gel forming a dense multicellular network (Day 8, C). ASC released from the CSM into collagen were more spindle in appearance (Day 2, D) which developed filopodias (Day 6 E). Over time they formed more elongated morphological structures stretching along fibril assemblies resembling cells that are associated with stromal tissues.

**[0029]** FIGS. **17**A-**17**F are images depicting Qdot 565 labeled ASC tracked after migration from chitosan microspheres after Day 6 into PEGylated fibrin and collagen gels. Epifluorescent images of Qdot 565 labeled ASC tracked after migration from the CSM into PEGylated fibrin (A-C) and collagen (D-F) after 6 days. ASC released from chitosan microspheres into both PEGylated fibrin and collagen could be tracked (A and D) over 6 days. Cells forming tubes-like structures (B) in PEGylated fibrin and striated morphologies (E) were colocalized with Qdot 565 (C and F).

**[0030]** FIGS. **18**A-**18**H are images depicting the bidirectional differentiation of ASC in the PEGylated fibrin—(ASC-CSM)—collagen gel constructs. ASC loaded in CSM exhibited matrix driven phenotypic changes into a fibroblast-like morphology in the collagen layer (A, C, E and G) and a tube-like morphology in the PEGylated fibrin layer (B, D, F and H) simultaneously. ASC started to migrate into both the gels on Day 3 (A and B) and proliferated as a fibroblast-like phenotype in collagen (C) and tube-like sprouts (D) in PEGylated fibrin on day 5. By day 7 the collagen layer showed an increase in fibroblast-like cells (E) which eventually populated the gels by day 11 (G). In the PEGylated fibrin layer the sprouts started to form long networks by day 7 (F) which formed complex networks by day 11 (H).

**[0031]** The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

**[0032]** While the present disclosure is susceptible to various modifications and alternative forms, specific example embodiments have been shown in the figures and are herein described in more detail. It should be understood, however, that the description of specific example embodiments is not intended to limit the invention to the particular forms disclosed, but on the contrary, this disclosure is to cover all modifications and equivalents as defined by the appended claims.

## DESCRIPTION

**[0033]** The present invention discloses a laminar construct for tissue-engineered dermal equivalents. By applying the teachings of this disclosure, a person of ordinary skill in the art would be able to create a multi-layered tissue culture from a single source of stem cells. The present teachings allow for the formation of both blood vessels and dermal connective tissue from a uniform cell population that may be seeded and spontaneously assemble into the desired layers without the need for long culture times that are necessary when multiple layers are cultured sequentially. This tissue culture is useful as a dermal equivalent or tissue-engineered skin.

**[0034]** In one embodiment, a laminar construct of the present disclosure comprises a hydrogel matrix and a population of mesenchymal stem cells (MSC). Stem cells are unique among cells that may be cultured because they are not constrained to a particular cell type. Rather, stem cells from a single source are capable of differentiating into a variety of distinct cell types depending on their environment and other external factors. External factors may include soluble signals, insoluble or matrix molecules or other cell types. Soluble signals include, but are not limited to, growth factors, hormones and bioactive small molecules. Matrix molecules include, but are not limited to, fibrous proteins such as collagen and elastin, adhesion proteins such as fibronectin and laminin as well as glycosaminoglycans such as hyaluronic acid or chondroitin sulfate either alone or complexed as a proteoglycan. In fact, a single source of stem cells may differentiate into multiple types of tissue depending upon the properties of the hydrogel matrix that surround an individual stem cell. In certain embodiments, the hydrogel matrix may